TAXONOMIC STATUS AND GENETIC STRUCTURE OF SPECKLED CHUBS (CYPRINIDAE: CF. *MACRHYBOPSIS AESTIVALIS*) IN THE ARKANSAS RIVER DRAINAGE

By

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Abstract

A recent study recognized two distinct morphological types of speckled chub (cf. *Macrhybopsis aestivalis*) in the Arkansas River drainage. The purpose of this study was to use protein electrophoresis to examine taxonomic status and genetic relationships of the two forms. A total of 196 speckled chub were collected from eight sites in Kansas and Oklahoma, seven in the Arkansas River drainage and one in the Wabash River in Indiana. The samples were assayed for genetic variation at 24 protein-encoding gene loci. The data were analyzed for insight into population structure. Phylogenetic relationships were examined on the basis of allele frequency parsimony and the distance-Wagner procedure.

The results, together with other studies, support recognition of two species in the Arkansas River drainage, *M. tetranema*, which is endemic to the drainage, and the more widespread *M. hyostoma*, which occurs elsewhere in the Mississippi River basin. The endemic species probably arose in the Ancestral Plains Stream, which drained much of western Kansas and Oklahoma and emptied into the Gulf of Mexico independently of the Mississippi River. Headward erosion of the Ancestral Arkansas River in the Pleistocene would have captured a portion of the Ancestral Plains Stream, bringing *M. tetranema* into contact with *M. hyostoma*. The genetic results, together with morphology, suggest that *M. hyostoma* in the Arkansas River drainage are intergrades with *M. tetranema*. The latter species, which has disappeared over approximately 90% of its geographic

range, now consists of two widely disjunct, geographically restricted populations. Aspects of genetic structure are discussed with respect to management implications for this declining species.

Introduction

Well resolved taxonomic information and knowledge of genetic structure is essential if management plans for declining or endangered species are to succeed (Frankel 1974, Daugherty et al. 1990, Echelle 1991). I use protein electrophoretic data to describe genetic variation in two currently recognized species of the declining speckled chub complex (Teleostei: Cyprinidae) in the Arkansas River drainage. Until recently, speckled chubs were treated as a single, wide ranging species, *Macrhybopsis aestivalis*, comprising a number of allopatric subspecies and ranging from the southeastern United States to the Rio Grande River in New Mexico and north to the 45th parallel (Wallace 1980). Speckled chubs of the Arkansas River were collectively referred to as the endemic subspecies *Macrhybopsis aestivalis tetranemus* (Miller and Robison 1973). However, a recent morphological study by David Eisenhour (pers. comm.) recognizes two species of speckled chub in the Arkansas River drainage, *M. tetranema,* which is endemic to the western portion of the area, and *M. hyostoma,* which is more widely distributed in the Mississippi River basin (Fig. 1).

As presently recognized, the endemic species of speckled chub in the Arkansas River drainage (*M. tetranema*) has disappeared from about 90% of its former range (Luttrell 1997). It persists in only two geographically restricted, widely separated areas,

the Ninnescah River of Kansas and the South Canadian River between Ute Reservoir in New Mexico and Merideth Reservoir in the Texas panhandle. The other species, *M. hyostoma*, has disappeared from about 55% of its former range in the Arkansas River drainage. It persists in the mainstem of the Arkansas River and downstream reaches of its tributaries between Kaw Reservoir and the McClellan-Kerr Navigation System in Eastern Oklahoma (Luttrell 1997).

My purposes were to describe geographic variation among populations of the two species in the Arkansas River drainage and to provide insight into the question of whether the area supports two species or only a single, morphologically plastic species. The question of how many species exist in the drainage is of obvious importance regarding management decisions (Avise 1994). I present a preliminary approach to this problem by addressing the question of monophyly for the populations recognized as *M. tetranema*. If they do not form a monophyletic group exclusive of other speckled chub populations in the basin, then the original, single-species taxonomy might be more appropriate.

The results indicate a very low level of genetic divergence among samples; however, it is generally unknown how much genetic divergence is reflected by morphological, behavioral, or ecological differences (Leary 1987). Correspondingly, phenotypic plasticity in fishes has made it difficult to distinguish between environmental and genetic influences on morphology, behavior and life history (Allendorf et al. 1987, Meffe and Vrijenhoek 1988). Several different, syntopically occurring morphotypes of cichlids from the Cuatro Cienegas basin of Mexico were considered separate species until genetic studies (Sage and Selander 1975) and breeding experiments demonstrated that

they were morphotypes of the same population (Kornfield et al. 1982). Similar results were obtained for two trophically divergent forms of the goodeid fish genus *Ilyodon* (Turner and Grosse 1980). In contrast, speciation can occur with little or no detectable genetic differences (Avise 1994). For example shovelnose sturgeon (*Scaphirhynchus platyrhynchus*) and pallid sturgeon (*S. albus*) are indistinguishable at 37 allozymic loci (Phelps and Allendorf 1983), although they are morphologically and ecologically divergent.

Knowledge of geographic patterns of genetic variation is critical for insight into management needs and priorities for declining species (Vrijenhoek et al. 1985, Avise 1994), yet such knowledge is available for only a small proportion of threatened fishes of western North America (Echelle 1991). Should the two populations of *M. tetranema* be managed as a single unit, or are they sufficiently different that management as separate units is more appropriate? Is there any evidence that one of the two populations is genetically introgressed by the other species in the drainage? Such questions are important for a number of management options, including reintroductions into areas of past occurrence, as suggested for *M. tetranema* by Luttrell (1997), and artificial transport among populations to maintain genetic diversity (Meffe and Vrijenhoek 1988).

Methods and Materials

Collections of 21-25 specimens of speckled chub were made between October 1995 and September 1996 from each of eight sites (Fig. 2) as follows (parentheses give abbreviations for the sites): (Nin) Ninnescah River at Kingman city park, Kingman

County, Kansas; (SF1) Salt Fork of the Arkansas River, N of Salt Fork at the highway 74 bridge, Grant County, Oklahoma; (SF2) Salt Fork of the Arkansas River at the mouth of the Chikaskia River, Kay County, Oklahoma; (Cim 1) Cimarron River 6.4 km W and 3.2 km S of Ames, Major County, Oklahoma; (Cim 2) Cimarron River at highway 77 bridge N of Guthrie, Logan County, Oklahoma; (SC1) South Canadian River 8.5 km SE of Logan, Quay County, New Mexico; (SC2) South Canadian River at the highway 385 bridge S of Boy's Ranch, Oldham County, Texas; and (Wab) Wabash River at the old dam 2.2 km SE of New Harmony, Posey County, Indiana. The collections included five samples of *M. hyostoma* (Cim 1, Cim 2, SF1, SF2, Wab) and three samples of *M. tetranema* (SC1, SC2, Nin).

Fish were collected by seining, placed in polypropylene tubes and frozen in liquid nitrogen for transport to the laboratory. Samples were stored at -60^0 C until processing. Epaxial muscle and a mixture of eye and brain tissue were homogenized separately with deionized water and centrifuged to obtain supernatant. Standard methods of protein electrophoresis (Murphy et al. 1991) were used to resolve 24 presumptive loci (Table 1) .

Except where otherwise noted, I used BIOSYS-1 (Swofford and Selander 1981) for all analyses. Percent polymorphism (P) and average heterozygosity (H, estimated from allele frequencies) were calculated for each sample. A locus was considered polymorphic if it exhibited more than one allele. Hardy-Weinberg equilibrium was tested with an exact significance test and Levene's correction for small sample size. Electrophoretic divergence among samples was examined in two ways: by computing Nei's (1975) genetic distance and by performing a principal components analysis,

(Wilkinson 1990) on the arcsine transformation of the square root of allele frequencies.

I used FREQPARS (Swofford and Berlocher 1987) to compare different tree topologies of relationships among the eight samples examined. FREQPARS computes the minimum amount of allele frequency change required by topologies provided by the user. Topologies tested included three groups: 1) trees produced by the unweighted pair group method of analysis (UPGMA) for Rogers (1972) genetic distance, Cavalli-Sforza and Edwards (1967) chord distance, and Nei's (1978) genetic distance, 2) distance-Wagner trees (mid-point rooting, multiple addition criterion, MAXTREE = 30) based on Rogers (1972) genetic distance and Cavalli-Sforza and Edwards (1967) chord distance, and 3) additional trees suggested by the geographic distribution of samples. Based on the distance-Wagner analysis, the sample from the Wabash River was designated the outgroup for the FREQPARS analysis.

F-statistics ($F_{(IS)}$, $F_{(IT)}$, and $F_{(ST)}$) were computed for seven combinations of samples: 1) all samples, 2) all samples of *M. hyostoma*, 3) all samples of *M. hyostoma* excluding the Wabash River sample, 4) all samples of *M. tetranema*, 5) all samples from the Arkansas River drainage, 6) all samples of *M. tetranema* from the South Canadian River plus the sample of *M. hyostoma* from the Wabash River, and 7) all samples of *M. tetranema* plus the Wabash River sample of *M. hyostoma*. I used contingency Chi-square analysis to test for allele frequency differences among populations at individual loci.

Results

Tables 2 and 3 show genotypes, average heterozygosity, and polymorphism for

each population of *M. tetranema* and *M. hyostoma*, respectively. Average heterozygosities (0.042-0.083) were similar to the values reported for other species of freshwater fish (Gyllensten 1985, Hartl 1988), while polymorphism (0.333-0.500) was somewhat greater than the average for fishes in general (Nevo 1978). Seventeen of the 24 loci examined were polymorphic. None of the 192 chi-square tests indicated significant deviation from Hardy-Weinberg expectations. Thus, there is no evidence for the presence of two reproductively isolated populations at my sample sites, an observation consistent with the small, generally negative $F_{(IS)}$ values computed for the various subsets of the samples (Table 4).

The genetic similarity between *M. tetranema* and *M. hyostoma* (Table 5) is comparable to values seen in comparisons of conspecific populations for a diversity of freshwater fishes (Avise and Aquadro 1982, Echelle et al. 1989). Correspondingly, there were no diagnostic differences between *M. tetranema* and *M. hyostoma*, and within species, no sample was diagnostically different from the others. Thus, my analysis of relationships is based only on allele frequency differences among samples.

Very little between-sample variation was indicated for the samples from the Arkansas River drainage ($F_{(ST)} = 0.033$). Adding the sample from the Wabash River to the Arkansas River samples resulted in a much higher index of subdivision ($F_{(ST)} = 0.125$). Maximum subdivision ($F_{(ST)} = 0.237$) occurred when the data set was reduced to include only the two samples of *M. tetranema* from the South Canadian River and the sample of *M. hyostoma* from the Wabash River. The set of samples consisting of *M. hyostoma* from the Arkansas River drainage is the only grouping that did not show significant

heterogeneity among populations (P = 0.223). The "global" fixation index $F_{(IT)}$ generally reflected the relative magnitude of $F_{(ST)}$ values for the various subsets of samples (Table 4).

Figure 3 presents a plot of sample scores on the first two axes resolved by principal components analysis of allele frequency variation. The first axis (PC I), which accounts for 32.1% of the total variance in allele frequencies, separates *M. hyostoma* from the Wabash River in Indiana from the remaining samples, all of which are from the Arkansas River drainage. Among samples from the Arkansas River drainage, those from the South Canadian River in New Mexico (SC1) and the Texas Panhandle (SC2) are the most divergent from *M. hyostoma* from the Wabash River (Wab). The remaining populations from the Arkansas River drainage are intermediate in PC I score, but shifted toward those for *M. tetranema* from the South Canadian River. The second axis (PC II), which accounted for 17.5 % of total variance, separated the Ninnescah River sample of *M. tetranema* from the remainder of the samples. The distribution of scores in Figure 3 suggests that, in the two-dimensional space represented by PC I and PC II, samples of *M. tetranema* form a cluster that is separate from *M. hyostoma*. However, this is not conclusive because of the small number of samples examined.

The distance-Wagner analyses of phylogenetic relationship produced a single tree from the matrix of Rogers genetic distances and a single tree from the matrix of Cavalli-Sforza chord distances. In both of these trees, *M. hyostoma* from the Wabash River was sister to a clade comprising all samples from the Arkansas River drainage. Within the latter clade, the two trees differed in placements of the samples of *M. hyostoma*. However, in both trees, the samples of *M. hyostoma* from the Arkansas River drainage formed a paraphyletic group in which some samples of *M. hyostoma* clustered more closely with *M. tetranema* than with other

samples of their own species. *Macrhybopsis tetranema* was monophyletic in both trees, forming a clade in which the population from the Ninnescah River was sister to a clade consisting of the two samples from the South Canadian River.

In a second approach to phylogenetic analysis, I first subjected four tree-topologies to the FREQPARS algorithm in a search for the shortest tree on the basis of allele-frequency parsimony. These four trees included the two described for the distance-Wagner analysis, the UPGMA tree derived from Nei's genetic distance, and a tree representing the UPGMA results based on Rogers distance and Cavalli-Sforza chord distance (the last two indexes resulted in identical UPGMA topologies). Unlike the distance-Wagner topologies, *M. tetranema* was paraphyletic in the UPGMA trees, with *M. tetranema* from the South Canadian River depicted as sister to a clade in which *M. tetranema* from the Ninnescah River was sister to *M. hyostoma* from the Arkansas River drainage.

The shortest of the resulting FREQPARS trees (Fig. 4) was the one based on the distance-Wagner topology derived from Rogers genetic distance. The FREQPARS length for this tree (8.66) was only slightly less than that for the next-shortest tree (length = 8.74), which was the one based on the distance-Wagner topology derived from Cavalli-Sforza chord distance. The FREQPARS lengths required by the two topologies derived from the UPGMA analyses were somewhat longer (9.09-9.10).

In a further attempt to find a shorter FREQPARS tree, I examined three additional topologies, each of which maintained the topology shown in Figure 4 for the samples of *M. tetranema*. In these trees, samples of *M. hyostoma* from the same river were grouped together in a single clade. This resulted in three lineages from the Arkansas River drainage: *M. tetranema*, *M. hyostoma* from the Salt Fork of the Arkansas River, and *M. hyostoma* from the

Cimarron River. The three possible arrangements of these lineages required FREQPARS tree-lengths (8.78 to 8.82) that were slightly longer than the original trees based on the distance-Wagner topologies (8.66 and 8.74).

A feature seen in both distance-Wagner trees and in the FREQPARS results from those trees and the three modified versions of Figure 4 is that branch-lengths for samples of *M. hyostoma* from the Arkansas River drainage were markedly shorter than those for the three samples of *M. tetranema*. Also, among the three samples of *M. tetranema*, the branch length for the sample from the Ninnescah River was shorter than those for the two samples from the South Canadian River.

Discussion

My analysis of population structure in speckled chubs from the Arkansas River basin provides no evidence for the heterozygote deficiencies expected from the Biological Species Concept for the co-occurrence of two species at the same site. These results could be an artifact of the small sample sizes (n = 21-25) and the low levels of genetic divergence between *M. tetranema* and *M. hyostoma*. Based on morphology, however, it appears that artificial reservoirs separate all extant populations of *M. tetranema* from *M. hyostoma* (Luttrell, 1997). Thus, there apparently is no opportunity for the "test of sympatry" regarding the question of whether or not the two forms represent good species under the Biological Species Concept.

Three lines of evidence support recognition of two species in the Arkansas River drainage. First, the two forms remain morphologically distinguishable, both historically

and at present (Eisenhour, pers. comm.; Luttrell, 1997), although *M. hyostoma* in the Arkansas River drainage shows evidence of morphological intergradation (Eisenhour, pers. comm.). Thus, the two forms qualify as species under the Evolutionary Species Concept, in which a species is an entity composed of organisms that maintains its identity from other such entities through time and over space and has its own independent evolutionary fate and historical tendencies (Mayden and Wood 1995). Second, placing the two forms into a single species would require the inclusion of a third, morphologically distinct form. Eisenhour's (pers. comm.) morphological analysis indicates that *M. australis*, which is endemic to the Red River drainage, is sister to *M. tetranema*. Combining these morphotypes under the same species name would effectively mask the existence of three morphologically recognizable entities that have maintained their identity through time. Finally, my phylogenetic analysis indicates that the extant populations of *M. tetranema* comprise a monophyletic group that excludes *M. hyostoma*, further supporting recognition of *M. tetranema* as a species separate from *M. hyostoma.* Additional data are required to address the question of monophyly for the wide-ranging *M. hyostoma*.

My analysis is compatible with the evidence from morphology (Eisenhour, pers. comm.) that, although morphologically distinct from *M. tetranema*, the populations of *M. hyostoma* in western reaches of the Arkansas River are intergrades between the two species. This is supported by the genetic intermediacy of the samples of *M. hyostoma* from the Arkansas River drainage (Fig. 3), and by the phylogenetic analysis. Inclusion of intergrades in a phylogenetic analysis based on either allele frequency parsimony or genetic distance would have two effects on the resulting phylogenetic tree: 1) the appearance of

paraphyly because of the topological placement of intergrades and 2) short branch lengths for the intergrades. Both of these features are evident for the Arkansas River samples of *M. hyostoma* (Fig. 4). The short branch lengths for intergrades are due to their intermediate genetic makeup, which FREQPARS would treat as the plesiomorphic condition present in the common ancestor and which would result in small genetic distances, thus short branch lengths, in results from the distance-Wagner procedure.

Eisenhour's (pers. comm.) phylogenetic analysis of morphology is consistent with the hypothesis that vicariant biogeography explains the presence of three species of speckled chub in the area encompassed by the Red and Arkansas River drainages. According to this hypothesis, the common ancestor of *M. tetranema* and *M. australis* would have occupied the Ancestral Plains Stream, which formed when Kansan glaciation diverted east-flowing streams southward to the Gulf of Mexico through present-day Kansas, Oklahoma, and Texas, independently of the Mississippi River (Metcalf 1966; Cross et al. 1986). Prior to the Sangamonian interglacial period (0.4-0.1 million years ago), the ancestral Arkansas and Red rivers eroded headward, capturing different portions of the Ancestral Plains Stream and diverting them separately into the Mississippi River (Cross et al. 1986). These events would have isolated populations that eventually evolved into *M. tetranema* and *M. australis* in, respectively, the Arkansas and Red river drainages, and would have established contact between both of these species and *M. hyostoma*, a form that would have evolved in the Ancestral Mississippi River Basin.

It is worth noting that, on the principal axis of genetic variation (PC I, Fig. 3), the Ninnescah River population of *M. tetranema* is intermediate between *M. tetranema* from

the South Canadian River in New Mexico and Texas and *M. hyostoma* from the Wabash River in Indiana. In addition, the historical distributions of the two species suggests greater opportunity for genetic introgression of the Ninnescah River population of *M. tetranema*. *Macrhybopsis hyostoma* once occurred in the lower Ninnescah River about 100 river-km downstream of my collection site, whereas the extant South Canadian River populations of *M. tetranema* were historically separated from *M. hyostoma* by more than 600 river-km (Figs. 1 and 2), most of which was sparsely inhabited by speckled chubs (Luttrell, 1997).

Further analysis, perhaps with non-recombining genetic markers such as mitochondrial DNA, would be required for a more conclusive answer to the question of whether the Ninnescah River population of *M. tetranema* has been introgressed by *M. hyostoma*. If the answer is yes, then this raises several controversial questions. How should the population be treated under the Endangered Species Act? Should it receive the same investment of resources as non-introgressed populations? Does it matter whether the introgression is anthropogenic or not? Such questions have generated considerable debate among conservation biologists (O'Brien and Mayr 1991; Gittleman and Pimm 1991; Phillips and Henry 1992). Because introgressed populations still carry locally adaptive genetic variation, we should not automatically discount them as valuable resources worthy of protection (Dowling and Childs 1992). Regarding red wolves, which are genetically introgressed by coyotes (probably as a result of human activity), O'Brien and Mayr (1991) argued that they deserve protection under the Endangered Species Act because they are the only available descendants of a historic wolf species. If introgression

is natural, as apparently is the case for speckled chubs, then it should not eliminate populations as candidates for re-introduction into areas of previous occurrence, unless there is evidence that the original population in the area of re-introduction was not introgressed. Thus, both of the remaining populations of *M. tetranema* are worthy of attention as the last remnants of a once more widespread species.

The $F_{(ST)}$ value for *M. tetranema* (0.025) suggests that approximately 97% ($1-F_{(ST)}$) of the existing genetic diversity in the species is contained in a single sample from either of the two remaining populations. This suggests an unusually high level of genetic cohesiveness compared with most other western species having disjunct populations (Echelle 1991). Based on collection records (Fig. 1), the two populations were historically isolated, even before construction of the reservoirs that now preclude gene flow (Luttrell 1997). From the management standpoint, the low level of geographic heterogeneity suggests that the two populations of *M. tetranema* might be treated as a single management unit. However, as previously mentioned, the Ninnescah River population might have diverged somewhat as a result of genetic introgression by *M. hyostoma*, a possibility that should be considered in management plans for the species.

It might be argued under the concept of the evolutionarily significant unit (ESU) that the two populations of *M. tetranema* be managed as separate units. An ESU is defined as "a population (or group of populations) that is 1) significantly reproductively isolated from other conspecific population units, and 2) represents an important component in the evolutionary legacy of the species" (Waples 1995). Thus, on the basis of the wide geographic separation of the two populations, the small but statistically

significant differences in allele frequencies, and the potential for local adaptations (through genetic introgression or other means), both South Canadian and Ninnescah populations qualify as ESUs. In other words the loss of one or the other of the two populations would represent a significant loss of the ecological-genetic diversity of the species. As emphasized by Waples, this is a key consideration in recognizing ESUs.

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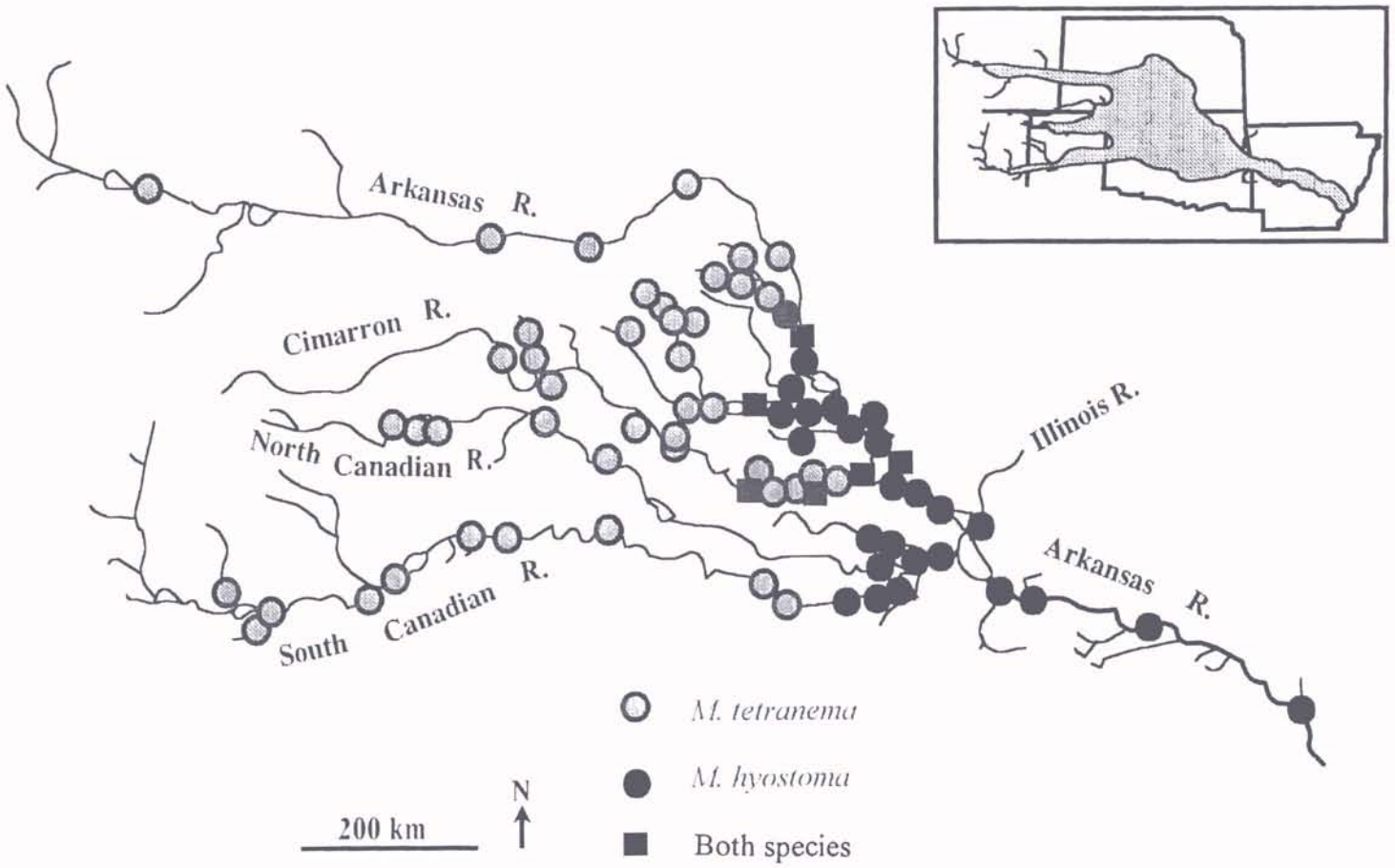

Figure 1. Historical distribution (1884-1990) of *M hyostoma* and *M. tetranema*.

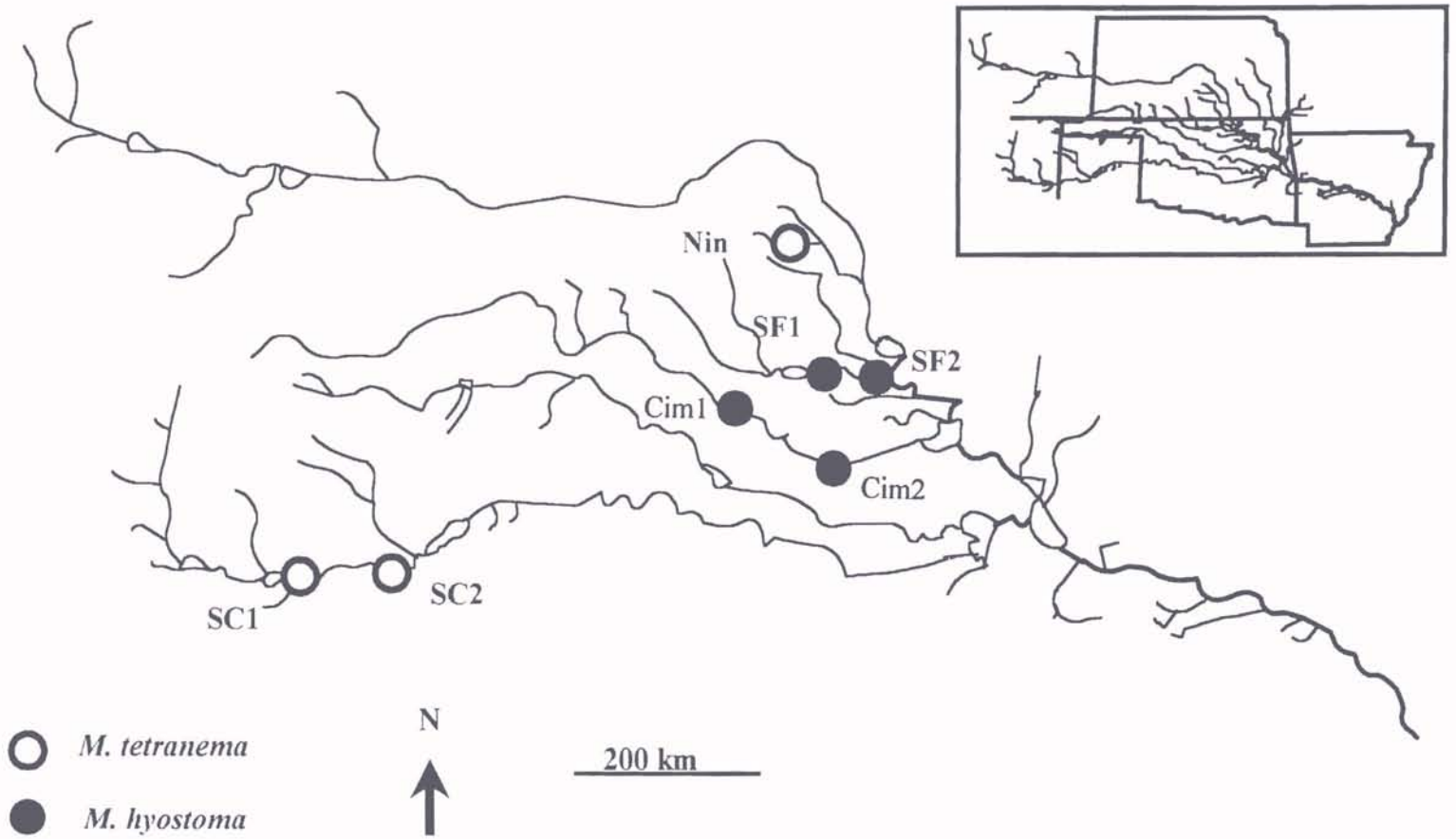

Figure 2. Sampling sites in the Arkansas River Basin
See text for the explanation of sample site designations.

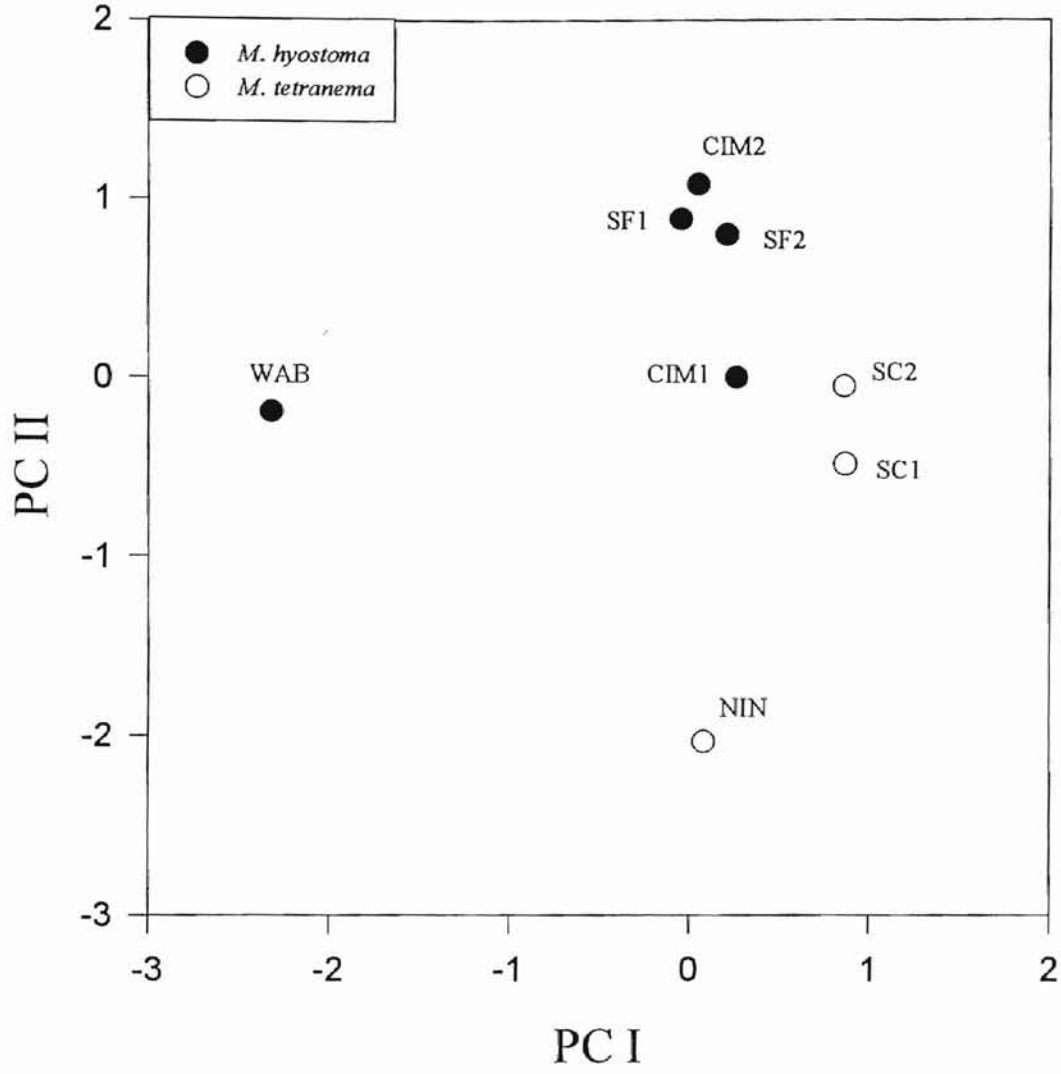

Figure 3. Principal components analysis of allele frequency variation. Sample abbreviations are those used in Figure 2.

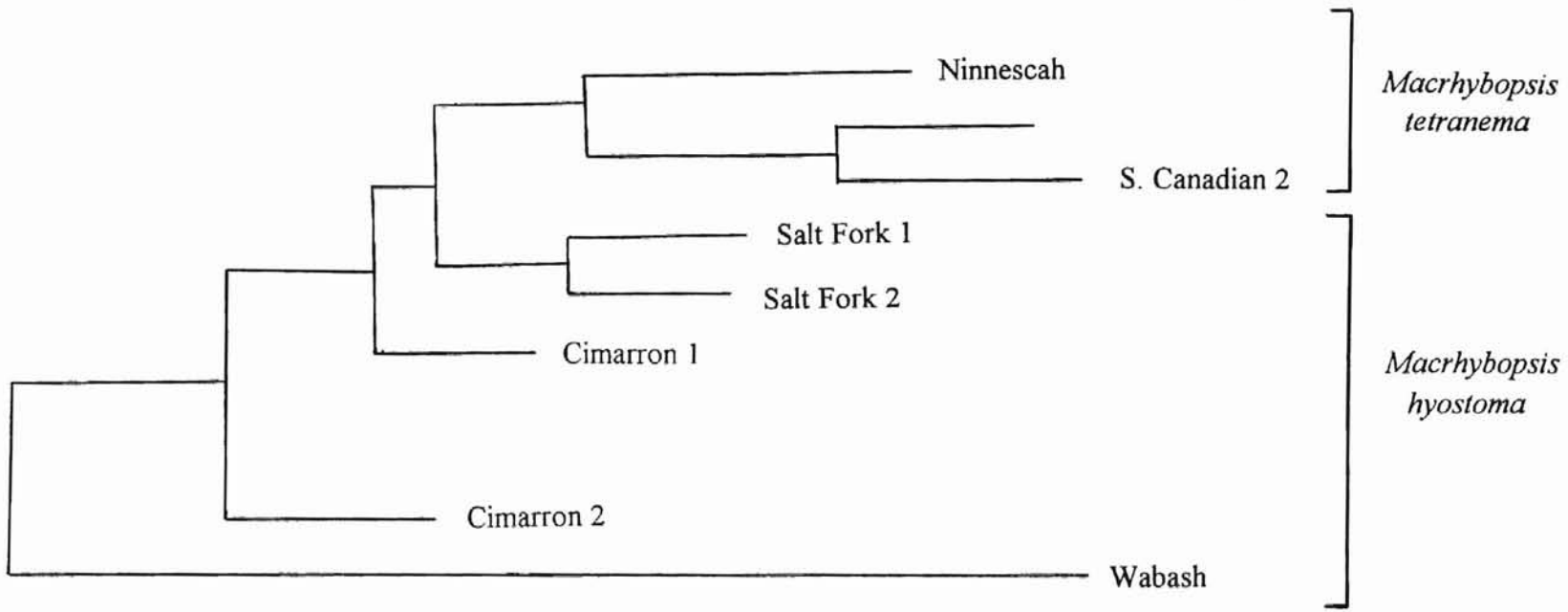

Figure 4. Freqpars tree for distance-Wagner topology derived from the matrix of Rogers genetic distances; total length = 8.66.

Table 1. Proteins, presumptive loci, and buffer systems used. Enzyme nomenclature follows the International Union of Biochemistry (1992). Locus designations follow Buth's 1984 reccommendations.

Enzyme or protein	Enzyme number	Locus	Tissue scored	Analytical system[1]
Adenylate kinase	2.7.4.3	Ak-A	muscle	3
Aspartate transaminase	2.6.1.1	s-Aat-A	eye-brain	1
Calcium binding protein-1	nonspecific	Cbp-1	muscle	1
Calcium binding protein-2	nonspecific	Cbp-2	muscle	1
Creatine kinase	2.7.3.2	CK-A	eye-brain	3
Creatine kinase	2.7.3.2	CK-B	eye-brain	3
Glyceraldehyde-phosphate dehydrogenase	1.2.1.9	Gapdh-A	eye	3
Glyceraldehyde-phosphate dehydrogenase	1.2.1.9	Gapdh-C	muscle	3
Glucosephosphate isomerase	5.3.1.9	Gpi-A	muscle	4
Glucosephosphate isomerase	5.3.1.9	Gpi-B	muscle	4
Isocitrate dehydrogenase	1.1.1.42	m-Idh-A1	eye-brain	1
Isocitrate dehydrogenase	1.1.1.42	m-Idh-A2	eye-brain	1
Isocitrate dehydrogenase	1.1.1.42	s-Idh-A	eye-brain	1
Lactate dehydrogenase	1.1.1.27	Ldh-A	eye-brain	2
Lactate dehydrogenase	1.1.1.27	Ldh-B	eye-brain	2
Malate dehydrogenase	1.1.1.37	s-Mdh-A	muscle	1
Malate dehydrogenase	1.1.1.37	s-Mdh-B	muscle	1
Malate dehydrogenase	1.1.1.37	m-Mdh-A	muscle	1
Malate dehydrogenase	1.1.1.40	m-Mdhp-A	eye-brain	1
Manose-6-phosphate isomerase	5.3.1.8	Mpi-A	eye-brain	2
Peptidase-A	3.4.-.-	Pep-A	muscle	1

Table 1., continued

Enzyme or protein	Enzyme number	Locus	Tissue scored	Analytical system[1]
Peptidase-B	3.4.-.-.	Pep-B	eye-brain	2
Phosphogluconate dehydrogenase	1.1.1.44	Pgd-A	muscle	3
Phosphoglucomutase	2.7.5.1	Pgm-A	muscle	1

[1]Analytical systems are as follows:

1) Electrode buffer and stock solution : 0.69 M Tris-Hydroxymethlaminomethane (= "Tris"), 0.16 M .016 M citric acid, pH 8.0; gel buffer: 1 volume stock, 28 volumes H_20 pH 8.0.

2) Stock solution: 0.90 M Tris, 0.50 M boric acid, 0.1 M disodium EDTA adjusted to pH 8.6; electrode solution: 1 volume stock, 6.9 volumes H_2O; gel buffer: 1 volume stock, 24 volumes H_2O.

3) Stock solution: 0.75 M Tris, 0.25 M citric acid, pH 7.0; anodal electrode buffer: 1 volume stock, 6 volumes H_2O; cathodal electrode buffer solution: 1 volume stock, 4 volumes H_2O; gel buffer: 1 volume stock, 19 volumes H_2O.

4) Electrode buffer and stock solutions: 0.223 M Tris, 0.86 M citric acid pH 6.0; gel buffer: 1 volume stock, 28 volumes H_2O.

All pH adjustments made with 10 N NaOH.

Table 2. Genotypes for polymorphic loci, average heterozygosity, and polymorphism for three populations of *Macrhybopsis tetranema*. Symbols for populations correspond with those in Figure 2.

Locus	Nin	SC1	SC2
s-Aat-A	100:100(21)	100:100(24)	100:100(25)
		100:81(1)	
Ak-A	100:100(21)	100:100(24)	100:100(23)
		121:100(1)	121:100(1)
Ck-b	100:100(19)	100:100(25)	100:100(24)
	100:92(2)		
Gpi-A	100:100(19)	100:100(22)	100:100(22)
	100:87(2)	100:80(1)	100:80(2)
		110:100(1)	100:87(1)
Gpi-B	100:100(13)	100:100(20)	100:100(25)
	1100:100(3)	700:100(1)	
	700:100(1)	-400:-400(1)	
	1100:-200(1)	100:-400(2)	
	100:-400(1)	100:-100(1)	
	100:-100(2)		
m-Idh-A1	100:100(20)	100:100(25)	100:100(25)
	100:95(1)		
m-Idh-A2	100:100(20)	100:100(25)	100:100(25)
	106:100(1)		
Ldh-B	100:100(15)	100:100(20)	100:100(24)
	350:100(6)	350:100(5)	350:100(1)
s-Mdh-A	100:100(21)	100:100(25)	100:100(25)
s-Mdh-B	100:100(21)	100:100(24)	100:100(25)
		115:100(1)	

Table 2., continued

Locus	Nin	SC1	SC2
m-Mdh-A	100:100(19) 100:73(1) 100:60(1)	100:100(25)	100:100(24) 126:100(1)
m-Mdhp-A	100:100(21)	100:100(24) 116:100(1)	100:100(25)
Mpi-A	100:100(19) 107:100(2)	100:100(20) 107:107(1) 107:100(4)	100:100(21) 107:100(3)
Pep-A	100:100(12) 100:88(5) 113:113(1) 113:88(1) 113:100(2)	100:100(15) 88:88(1) 100:88(5) 100:72(2) 113:100(2)	100:100(15) 113:100(1) 100:72(2) 100:88(7)
Pep-B	100:100(21)	100:100(25)	100:100(24) 121:100(1)
Pgd-A	100:100(16) 100:43(1) 100:75(3) 75:75(1)	100:100(25)	100:100(25)
Pgm-A	100:100(16) 100:85(1) 100:88(4)	100:100(16) 118:100(5) 100:88(1) 100:85(3)	100:100(20) 118:100(2) 118:88(1) 100:88(2)
H	0.083	0.065	0.042
P	0.458	0.417	0.333

H = average heterozygosity per sample.

P = polymorphism per sample.

Alleles are designated by the proportional migration of the product relative to that of the most common allele.

Table 3. Genotypes for polymorphic loci, average heterozygosity, and polymorphism for five populations of *Macrhybopsis hyostoma*. Symbols for populations correspond with those in Figure 2.

Locus	Cim1	Cim2	SF1	SF2	Wab
s-Aat-A	100:100(25)	100:100(24) 100:89(1)	100:100(24)	100:100(25)	100:100(23) 100:89(1)
Ak-A	100:100(23) 121:100(1) 100:64(1)	100:100(24) 121:100(1)	100:100(24) 100:64(1)	100:100(23) 121:100(1) 100:64(1)	100:100(25)
Ck-b	100:100(25)	100:100(25)	100:100(25)	100:100(24)	100:100(25)
Gpi-A	100:100(22) 100:87(2) 100:80(1)	100:100(20) 110:100(5)	100:100(23) 110:100(1) 100:80(1)	100:100(20) 110:87(1) 110:80(1) 100:80(1) 100:87(1) 110:100(1)	100:100(24) 110:100(1)
Gpi-B	100:100(19) 1100:100(3) 1100:1100(1) 700:-100(1) 700:100(1)	100:100(20) 1100:100(3) 100:-100(2)	100:100(12) 1100:100(8) 1100:-600(1) 700:100(1) 900:100(1) 1100:-100(1) 100:-200(1)	100:100(18) 1100:1100(2) 1100:100(5)	100:100(19) 100:-200(2) 100:-600(1) 700:100(2) 1100:100(1)

Table 3., continued

Locus	Cim1	Cim2	SF1	SF2	Wab
m-Idh-A1	100:100(25)	100:100(25)	100:100(25)	100:100(24) 100:89(1)	100:100(24) 100:89(1)
m-Idh-A2	100:100(25)	100:100(25)	100:100(24) 100:94(1)	100:100(25)	100:100(22) 106:100(3)
Ldh-B	100:100(16) 350:100(8) 350:350(1)	100:100(14) 350:100(10) 100:50(1)	100:100(17) 350:100(7) 350:350(1)	100:100(18) 350:100(7)	350:100(10) 350:350(15)
s-Mdh-A	100:100(25)	100:100(24) 100:87(1)	100:100(25)	100:100(25)	100:100(17) 100:87(8)
s-Mdh-B	100:100(23) 115:100(2)	100:100(25)	100:100(25)	100:100(25)	100:100(25)
m-Mdh-A	100:100(25)	100:100(24) 126:100(1)	100:100(25)	100:100(23) 126:100(2)	100:100(25)
m-Mdhp-A	100:100(25)	100:100(24) 116:100(1)	100:100(25)	100:100(24)	100:100(25)

Table 3., continued

Locus	Cim1	Cim2	SF1	SF2	Wab
Mpi-A	100:100(18) 104:100(1) 107:100(3)	100:100(25)	100:100(25)	100:100(21) 107:107(1) 112:107(1)	100:100(24) 100:90(1)
Pep-A	100:100(14) 113:100(8) 113:113(1) 100:88(1) 113:88(1)	100:100(16) 113:113(2) 113:100(6) 113:72(1)	100:100(13) 113:100(8) 113:88(1) 100:88(2) 113:72(1)	100:100(13) 113:113(1) 113:100(8) 113:88(1) 88:88(1) 100:88(1)	113:113(11) 120:113(1) 113:100(13)
Pep-B	100:100(25)	100:100(25)	100:100(25)	100:100(24) 121:100(1)	100:100(24) 100:90(1)
Pgd-A	100:100(22) 125:100(1) 100:43(1)	100:100(21) 100:43(2) 100:90(2)	100:100(22) 100:90(2) 100:43(1)	100:100(22) 100:90(1)	100:100(19) 140:100(2) 100:75(1) 125:100(2)
Pgm-A	100:100 (24) 118:100(1)	100:100(21) 100:85(4)	100:100(23) 118:100(1) 100:85(1)	100:100(23) 100:88(1) 100:85(1)	100:100(22) 100:88(1) 100:85(1) 118:100(1)

Table 3., continued

	Cim1	Cim2	SF1	SF2	Wab
H	0.066	0.067	0.064	0.065	0.078
P	0.375	0.458	0.333	0.417	0.500

H = average heterozygosity per sample.

P = polymorphism per sample.

Alleles are designated by the proportional migration of the product relative to that of the most common allele.

Table 4. F-statistics for various combinations of samples of *Macrhybopsis hyostoma* and *M. tetranema*. Asterisks signify statistically significant among-sample heterogeneity in allele frequencies: *, $p<0.025$; **, $p<0.0001$.

Sample grouping	$F_{(IS)}$	$F_{(IT)}$	$F_{(ST)}$
M. tetranema and *M. hyostoma*			
All samples	-0.026	0.096	0.119**
Arkansas River basin	0.001	0.033	0.033**
Wabash *M. hyostoma* plus all *M. tetranema*	-0.054	0.147	0.191**
Wabash *M. hyostoma* plus S. Canadian *M. tetranema*	-0.079	0.177	0.237**
M. hyostoma only			
All samples of the species	-0.038	0.091	0.125**
Arkansas River basin	0.003	0.016	0.013
M. tetranema only	-0.003	0.022	0.025*

Table 5. Nei's genetic identity among samples of *M. tetranema* and *M. hyostoma*. Sample abbreviations correspond to those given in Figure 2.

	M. tetranema			*M. hyostoma*				
Sample	Nin	SC1	SC2	Cim1	Cim2	SF1	SF2	Wab
Nin								
SC1	0.999							
SC2	0.998	1.000						
Cim1	1.000	0.998	0.997					
Cim2	0.999	0.998	0.997	1.000				
SF1	1.000	0.997	0.996	1.000	1.000			
SF2	1.000	0.998	0.997	1.000	1.000	1.000		
Wab	0.966	0.960	0.954	0.972	0.973	0.971	0.969	

VITA

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Candidate for the Degree of

Master of Science

Thesis: TAXONOMIC STATUS AND GENETIC STRUCTURE OF SPECKLED CHUBS (CYPRINIDAE: CF. *MACRHYBOPSIS AESTIVALIS*) IN THE ARKANSAS RIVER DRAINAGE

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Professional Memberships: American Fisheries Society